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GABAergic Effects of Some Food Extracts Via Inhibition of GABA-Transaminase

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Abstract

Objective: GABAergic system is a target for various groups of medications including sedatives, anxiolytics, muscle relaxants, antidepressants and antiepileptics. Several foods or food ingredients are able to affect the GABAergic system by the inhibition of the γ-aminobutyric acid (GABA) degrading enzymes including the GABA-transaminase and succinate semialdehyde dehydrogenase. The purpose of this study to investigate the inhibitory effects of tea (*Camellia sinensis*), coffee (*Coffea arabica L.*), peppermint (*Mentha piperita L.*), thyme (*Thymus vulgaris L.*), and cinnamon (*Cinnamomum zeylanicum*) on GABA degrading enzymes.

Methods: The inhibition of the GABA-T by aqueous extracts of tea (*Camellia sinensis*), coffee (*Coffea arabica L.*), peppermint (*Mentha piperita L.*), cinnamon (*Cinnamomum zeylanicum*), and thyme (*Thymus vulgaris L.*) was investigated using a fluorometric microplate enzyme assay. Dose-dependent inhibition of the GABA-degrading enzymes was attained by all the food extracts tested. For determination of the IC50 values of the extracts (± 95 % CI), a linear regression was performed using Origin® (Origin® 2015G von Origin Lab Corporation, Northampton, MA 01060 USA).

Results: The aqueous extract of black tea presented the strongest inhibitory activity with an IC50-value (half maximal inhibitory concentration) of 13.0 (11.0-15.3) µg/mL. The tested food extracts were successful in inhibiting the GABA-degrading enzymes even at low concentrations.

Conclusion: In conclusion, the selected food extracts could serve as natural inhibitors for GABA-degrading enzymes thus, they could increase the GABA concentration in the brain.

Key words: γ-Amino butyric acid, GABA degrading enzymes, enzyme inhibition, black tea, thyme

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Introduction

GABA is formed from glutamate-by-glutamate decarboxylase, which is a pyridoxal phosphate-dependent enzyme, and has two isoforms (65-kDa GAD and 67-kDa) in the human organism (1-5). The degradation of GABA (Fig. 1) occurs via a transamination reaction catalyzed by the GABA transaminase (GABA-T). GABA-T catabolizes GABA to the succinic semialdehyde that is either oxidized to the succinate by a reductase enzyme, the succinic semialdehyde dehydrogenase (SSA-DH; EC 1.2.1.24)) or reduced to the γ-hydroxybutyrate by the

succinic semialdehyde reductase (SSA-R; EC 1.1.1.61)) (6-8).

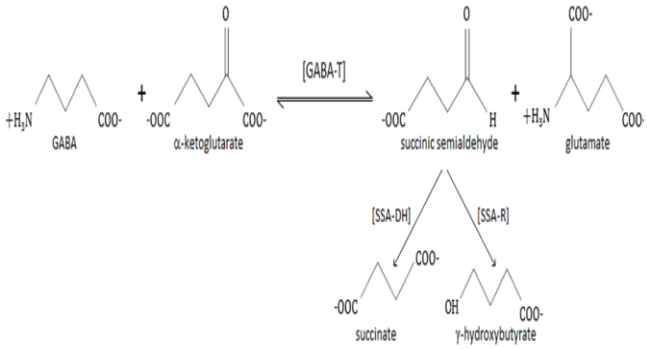

Figure 1. Schematic overview of degradation of neurotransmitter GABA; GABA-T: GABA transaminase; SSA-DH: Succinic semialdehyde dehydrogenase; SSA-R: Succinic semialdehyde reductase (6).

Low levels of GABA in the brain are associated with some neurophysiological diseases such as epilepsy, depression, panic disorders, anxiety disorders or sleep disorders (9-15). In the human study, Wood et al. (13), reported that epileptic patients have significantly low levels of GABA in cerebrospinal fluid. In the brain of depressed patients, a significantly decreased GABA concentration was also detected (14). Since the permeability of the blood-brain barrier for GABA is very low (16, 17), GABA supplementation cannot lead to an increase in GABA levels in the brain. For example, no effect on the GABA concentration in the brain of rats could be demonstrated by intraperitoneal injection of GABA (18). However, the increasing of GABA concentration is possible by the inhibition of GABA degradation (19-23).

GABA-T is a target for antiepileptic drugs, which can increase GABA levels in the brain via inhibition of GABA-T, thereby eliciting an antiepileptic or anticonvulsant effect (19, 21). Some anticonvulsants such as vigabatrin, a vinyl derivative of GABA, act as a competitive inhibitor of GABA-T (7). In addition, the inhibitory effects of several anxiolytic plants on GABA-T have been reported (24-26). Since the inhibition of GABA-T causes an increase in GABA level in the brain, there is a growing interest in the inactivation of GABA-T by foods.

Although the anxiolytic, sedative, hypnotic, and calming effects of some food plants and their ingredients have been studied in numerous studies, their physiological targets have not been completely elucidated. For example, animal model studies demonstrated that epigallocatechin gallate, a major catechin found in green tea (*Camellia sinensis*), acts as sedatives and as hypnotics in the brain (27). Coffee (*Coffea arabica L*) is consumed because coffee reduces stress, drowsiness, and neuralgia (28). Peppermint (*Mentha piperita L.*) has a relaxation effect on the muscular actions and secretory processes of the gastrointestinal tract, analgesic, and anesthetic effects in the central and peripheral nervous system (29). Some Thymus spp. are used to produce antiseptic, antispasmodic, antioxidative, and sedative effects (30). Cinnamon (*Cinnamomum zeylanicum*) exhibited anti-depressant and anti-anxiety like effect in mice (31). The present study evaluated the effects of tea, coffee, peppermint, thyme, and cinnamon on GABA-T to explain their physiological action mechanism in the central nervous system.

Methods

Chemicals

GABA, GABase from *Pseudomonas fluorescens*, and α-ketoglutarate were purchased from Sigma-Aldrich (Taufkirchen, Germany). β-nicotinamide adenine dinucleotide phosphate (NADP) disodium salt was obtained from AppliChem (Darmstadt, Germany) and vigabatrin from British Pharmacopoeia Commission Laboratory (London, United Kingdom).

Plant Material and Preparation of Extracts

Peppermint leaves (*Mentha piperita L.*), thyme leaves (*Thymus vulgaris L.*), black tea leaves (*Camellia sinensis*) and cinnamon powder (*Cinnamomum zeylanicum*) were obtained in crushed form from a local pharmacy. Roasted coffee beans (*Coffea arabica L.*) were finely ground before extraction. 100 mL of boiling water were added to 2.5 g of the test material. The mixtures were stirred (60 min at 90 °C) and filtered through filter paper. The solvents in the filtrates were removed by freeze-drying (Lyophilizer Savant Novalyphe NL150). After lyophilization, the samples were kept at -20 °C and dissolved in water (1 mg/mL) before use.

Fluorometric Microplate Enzyme Assay

The activity of GABAse composed of GABA-T and SSADH was determined spectrophotometrically according to literature (32) with some modifications. The reaction mixture contained potassium pyrophosphate buffer (150 mM, pH= 8.0), α-ketoglutarate (228,5 mM), GABA (873 mM), and GABAse (1,5-1,7 units/mg). The extracts were added to the reaction mixture and preincubated (30 min at 37 °C). After preincubation, NADP+ (26.1 mM) was added to the mixture and incubated (30 and 60 min at 37 °C). GABA-T activity was monitored by

measuring the absorbance changes at 340 nm due to the reduction of NADP+. The absorbance was measured using a microplate spectrophotometer (µQuant BioTek). Vigabatrin, an inhibitor of GABA-T, was used as the positive control. Water was used instead of the inhibitor for the negative control.

Statistical analysis

For determination of the IC50 values of the extracts (± 95 % CI), a linear regression was performed using Origin® (Origin® 2015G von OriginLab Corporation, Northampton, MA 01060 USA).

Results

Each food extract was tested for its inhibitory activity at various concentrations (4-180 µg/mL). The activity of GABA-T decreased with increasing concentrations of black tea extract (Fig. 2a). Similarly, all other plant extracts exhibited inhibitory effects on GABA-T activity in a dose-dependent manner (Fig. 2 b, c, d, e). At the concentration of 18 µg/mL, cinnamon powder, coffee beans, peppermint leaves and thyme leaves extracts led to very weak inhibition (5.1 %, 8 %, 12.8 % and 21.5 % inhibition of GABA-T, respectively), whereas the extract of black tea induced the strongest inhibitory effect (56.6 % inhibition). When black tea extract was tested at the lower concentrations of 18 µg/mL, it also showed high inhibition indicating that it is the most potent inhibitor. Black tea showed 74 % inhibition at the highest concentration tested (27 µg/mL). Interestingly, the extracts of thyme and coffee showed similar GABA-T inhibition effects (65 % inhibition) at the concentration of 108 µg/mL. Compared to thyme and coffee in the same concentration (108 µg/mL), the extracts of peppermint and cinnamon induced lower activity (55 % and 34 % inhibition, respectively). The cinnamon extract exhibited only 55% inhibition at the highest concentration used (180 µg/mL).

The inhibitory effect of each food extract was evaluated by determining its respective IC50 values (the half-maximal inhibitory concentration). The IC50 values of extracts ranged between 13 and 174.2 µg/mL (Table 1). The strongest inhibitory effect was seen with the aqueous extract of black tea (IC50=13.0 µg/mL). The extracts of coffee, peppermint and thyme showed fifty percent inhibition at concentrations lower than 100 µg/mL. On the other hands, cinnamon extract was used at a much higher concentration (174.2 µg/mL) to inhibit 50 % of the enzyme.

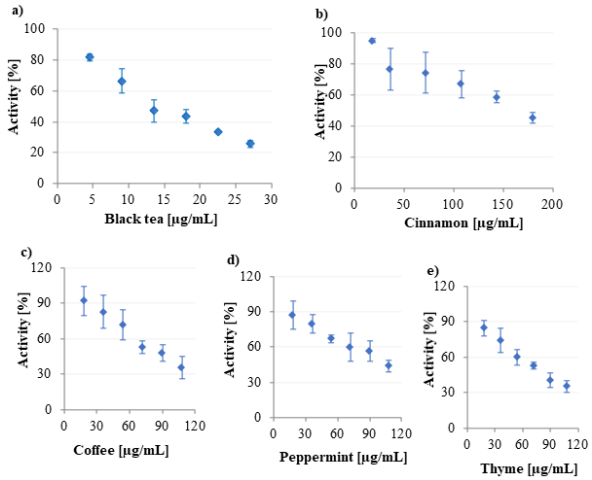

Figure 2. Dose response curve for the effect of the aqueous extract of black tea (a), cinnamon (b), coffee (c), peppermint (d), and thyme (e) on GABA-T activity expressed as percent of control. Values are the mean ± SD from triplicate analysis

Table 1. IC50 of aqueous extracts on GABA-T

Botanical	IC_{50} (with 95 % confidence interval) [µg/mL]
Black tea	13.0 (11.0-15.3)
Thyme	72.6 (60.9-86.5)
Coffee	83.9 (71.1-98.9)
Peppermint	97.5 (81.3-116.9)
Cinnamon	174.2 (135.4-224.2)

Discussion

Vigabatrin, which was designed to increase the amount of GABA in the central nervous system (7), binds as an inhibitor selectively and irreversibly at the active site of GABA-T (33). As the positive control, vigabatrin was used at the concentration of 2.7 mM (348.7 µg/mL) in the enzyme assay to confirm that detectable in vitro stimulation of total GABA-T inhibition could be measured under our experimental conditions. The food extracts showed their inhibitory effects in the concentrations of 4-180 µg/mL despite using lower concentrations than vigabatrin.

Awad et al. (24) demonstrated that the inhibitory effects of the aqueous and ethanolic extracts of traditionally used anxiolytic botanicals (*Centella asiatica, Eschscholtzia californica, Humulus lupulus, Hypericum perforatum, Matricaria recutita, Melissa officinalis, Passiflora incarnata, Piper methysticum, Scutellaria lateriflora*, and *Valeriana officinalis*) on GABA-T. They reported that IC50 values of the anxiolytic botanicals ranged from 350 to > 4000 µg/mL and the aqueous extract of M. officinalis showed the greatest inhibition of GABA-T with an IC50 of 350 µg/mL (24). Similarly, the ethanolic extracts of 34 traditional Q'eqchi' Maya plants, which used to treat some mental diseases such as

epilepsy and anxiety, were tested by Awad et al. (2009) for activity in the GABA-T. The IC50 value of the most active plant extract was 420 µg/mL (34). Compared to these anxiolytic botanicals, the aqueous food extracts tested in this study exhibited a higher inhibitory activity against GABA-T with very low IC50-values (13.0-174.2 µg/mL). Therefore, it can be concluded the aqueous food extracts (tea, thyme, coffee, peppermint, and cinnamon) may be more useful in the treatment of epilepsy and anxiety than the anxiolytic botanicals reported by Awad et al. 2007 and 2009.

Tea is consumed all over the world to relieve stress, drowsiness, and neuralgia (35). It is rich in flavonoids that have antioxidant, antitoxic, anticarcinogenic, antispastic, and antiviral activities. A study in the chick brain showed that epigallocatechin gallate, a major flavonoid of tea, acts as sedatives and as hypnotics in the brain, thereby moderating the acute stress response (27). Additionally, the anxiolytic effect of epigallocatechin gallate on mice has been reported (36). Furthermore, a human study described the anxiolytic effect of L-theanine, which is an important bioactive compound of tea (37). Like tea, coffee is consumed because coffee reduces stress (28). Peppermint, one of the most popular single ingredient herbal teas, has a relaxation effect on the muscular actions and secretory processes of the gastrointestinal tract, analgesic, and anesthetic effects in the central and peripheral nervous system, immunomodulating actions and chemo preventive potential (29). The primary constituent of peppermint oil is menthol (38) which has analgesic (39) and anesthetic activities (40). Thymol, a structural analogue of menthol, is found in thyme that is used to produce the antiseptic, antispasmodic, antioxidative, and sedative effects (30). Cinnamon showed an anti-depressant-like and anti-anxiety like effects in animal studies (31).

Conclusion

In conclusion, since low levels of GABA in the brain are associated with some neurophysiological diseases such as epilepsy, depression, panic disorders, anxiety disorders, or sleep disorders (9-15), it can be deduced that these food plants (tea, thyme, coffee, peppermint, and cinnamon) may produce their reported sedative, anti-depressant, anti-anxiety or anxiolytic effects by increasing the level of GABA due to inhibition of GABA-T.

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